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Studies on the free-living protozoan fauna of estuaries in the coastal zone of Tamil Nadu, India

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Abstract

A study was conducted to explore and document the free living protozoan diversity in estuaries in coastal zone of Tamil Nadu for the first time. The study covered the estuaries, *viz*., Arasalar, Vettar, Kaduviyar, Kollidam, Vellar, Marakkanam, Pulicat and Kaliveli of coastal zone of Tamil Nadu and resulted in the occurrence of 58 species of free-living protozoa including testate amoebae, tintinnids and foraminiferans from different estuaries coming under 9 genera and 6 families. All the species are new records from these estuaries and 5 species constituted as new records to India, *viz*., *Arcella excavata* Cunningham 1919, *Centropyxis delicatula* Penard 1902, *Centropyxis discoides* Penard 1902, *Difflugia binucleata* Penard 1902 and *Difflugia geosphaira* Ogden 1991.

Keywords: Bioindicator, New Records, Protozoa, Testate Amoebae, Tintinnids

Introduction

Estuaries are one of the greatest natural resources on earth and are a crucial source of biodiversity at the land-sea interface, providing valuable resources to a variety of species (Elling *et al.*, 2016). They are characterized by rich taxonomic diversity of organisms, high number of endemic species, and extremely high productivity. On the other hand, wetlands are among the most endangered places on the planet. Urbanization, agricultural development and resource extraction pressures have led to a worldwide decline in estuarine habitat, making these coastal ecosystems some of the most heavily used and threatened natural systems in the world (Lotze *et al.*, 2006). A number of species whose survival depends mainly on the wetlands are in danger of extinction.

Protozoa are microscopic unicellular eukaryotes that have a relatively complex internal structure and carry out complex metabolic activities. Protozoa are found in every possible habitat especially in all types of wetlands. The amoeboid protists or testacids are considered one of the largest and most diverse assemblages in the tree of eukaryotes (Pawlowski & Burki, 2009). These rhizopods are characterised by tests or shells which cover the entire body of the organisms for locomotion and collection of food. Previously all amoeboid protists were placed in Sarcodina. In the current eukaryotic tree sarcodina is a polyphyletic group with Amoebozoa and Rhizaria containing the highest number of species (Foissner, 1987; Adl *et al.*, 2005; Cavalier-Smith, 2004; Pawlowski & Burki, 2009). Testate amoebae or testaceans are one of the most abundant moss dwelling micro faunal components. It is known that testate amoebae are one of the basic groups of free-living protozoa, residentially present in water, soil and moss habitats. The fossil record in the form of shells in sedimentary rocks shows that protozoa were present in the pre-cambrian era. It is known that testate amoebae are one of the basic groups of free living protozoa, residentially present in water biocenoses. Testate amoebae, as primary destructors of cellulose and lignin, take an active part in diverse biological processes in water ecosystems. Testate amoebae are widespread and common inhabitants of water bodies. In them they occur in many types of biotopes. Testaceans are abundant and frequent component of the benthos which inhabited various types of sediment. They are well presented in the hydrophytes and submerged vegetation. Many forms of testate amoebae are occurring among plankton samples even though they are not typically planktonic (Lee *et al.*,

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2002; Roe & Patterson, 2006; Wall *et al.*, 2010; Lara *et al.*, 2016). They are considered as reliable bioindicators and biomonitors of environmental gradients, changes or pollution in terrestrial (Mitchell *et al.*, 2008), moss and limnetic habitats (Wall *et al.*, 2010). As their shells are well preserved over time in sediments and peat they are commonly used for quantitative palaeoecological studies (Charman, 2001). Despite a wide span of protozoological research carried out in different parts of India estuarine protozoa has until recently remained a practically uninvestigated group of the animal world. The objective of the study was to explore and document the protozoan diversity especially the testate amoebae diversity in various estuaries of Tamil Nadu which was not studied earlier. The only published records on the testate amoebae diversity in Tamil Nadu is the study of Bindu *et al.* (2014) from seagrass ecosystems of Palk Bay in which they reported 21 species of testate amoebae under 7 genera and 6 families.

Material and Methods

The survey covered 8 estuaries in the coastal zones of Tamil Nadu *viz*., Arasalar (10.91307°N 79.84638°E), Vettar (10.82658°N 79.85039°E), Kaduviyar (10.45100°N 79.50493°E), Kollidam (Coleroon) (11.35476°N 79.81268°E), Vellar (11.48493°N 79.77431°E), Marakkanam (12.23452°N 79.58075°E), Kaliveli (12.27306°N 80.01071°E) and Pulicat (13.406910 N 80.313620 E) estuaries during the period from 2013 to 2015. All the samples were collected by Bindu L. and party during the above mentioned study period. Water samples were collected by using plankton net of mesh size 20µm by towing from a boat at least for 2 hours and transferred to bottles. These samples from various lakes with flocculent matter and bottom oozes were kept in the laboratory for some days for considerable increase in protozoa population. Also water samples were collected with aquatic vegetation such as algae, water weeds and also bottom sediments, ooze, flocculent matter. These were collected in wide mouthed jars and were brought to the laboratory and kept it opened for a few days to increase the population of protozoans. These samples were examined under the light microscope Model Nikon 50i from time to time. The free-living protozoans occurring in them were isolated and examined in living condition by keeping them in a drop of natural medium. For making permanent slides of testacean rhizopods empty tests which are frequently found in abundance were isolated and were then air dried after two or three washings in absolute alcohol and mounted in DPX. Each slide was registered and deposited in the National Zoological collections of Marine Biology Regional Centre, Zoological Survey of India, Chennai.

The inventory list of the testate species is presented here along with specimen particulars following the classification of Adl *et al.* (2005). The descriptions of the specimens have also been provided.

Results

The study revealed a total of 58 species span over 9 genera and 6 families of free-living protozoans comprising of testate amoebae, tintinnids and foraminiferans recorded from various estuaries of Tamil Nadu. All the species are recorded for the first time from the studied estuaries and 25 species became new records to Tamil Nadu and 5 species *viz*., *Arcella excavata* Cunningham 1919, *Centropyxis delicatula* Penard 1902, *Centropyxis discoides* Penard 1902, *Difflugia binucleata* Penard 1902 and *Difflugia geosphaira* Ogden 1991 constituted new records to India. The systematic account of the species studied is provided hereunder.

Systematic Account

Phylum AMOEBOZOA (Luhe, 1913)

Class LOBOSEA Carpenter, 1861

Order ARCELLINIDA Kent, 1880

Suborder ARCELLINA Haeckel, 1884

Family ARCELLIDAE

1. *Arcella arenaria* Greeff, 1866

Material examined: Mi-140, 3 ex., Kaduviyar estuary, 8.i.2013.

Diagnosis: Shell more or less circular; dark brown in colour; aperture small and irregularly circular in shape; around the aperture many small pores present; diameter of the shell 90 µm and diameter of the aperture 21µm.

Distribution in India: Assam, Himachal Pradesh, Tamil Nadu (present record).

2. *Arcella catinus* Penard, 1980

Material examined: Mi-114, 4 ex., Pulicat lake, 31.vi.2012; Mi-127, 6 ex., Coleroon estuary, 04.vii.2012; Mi-134, 2

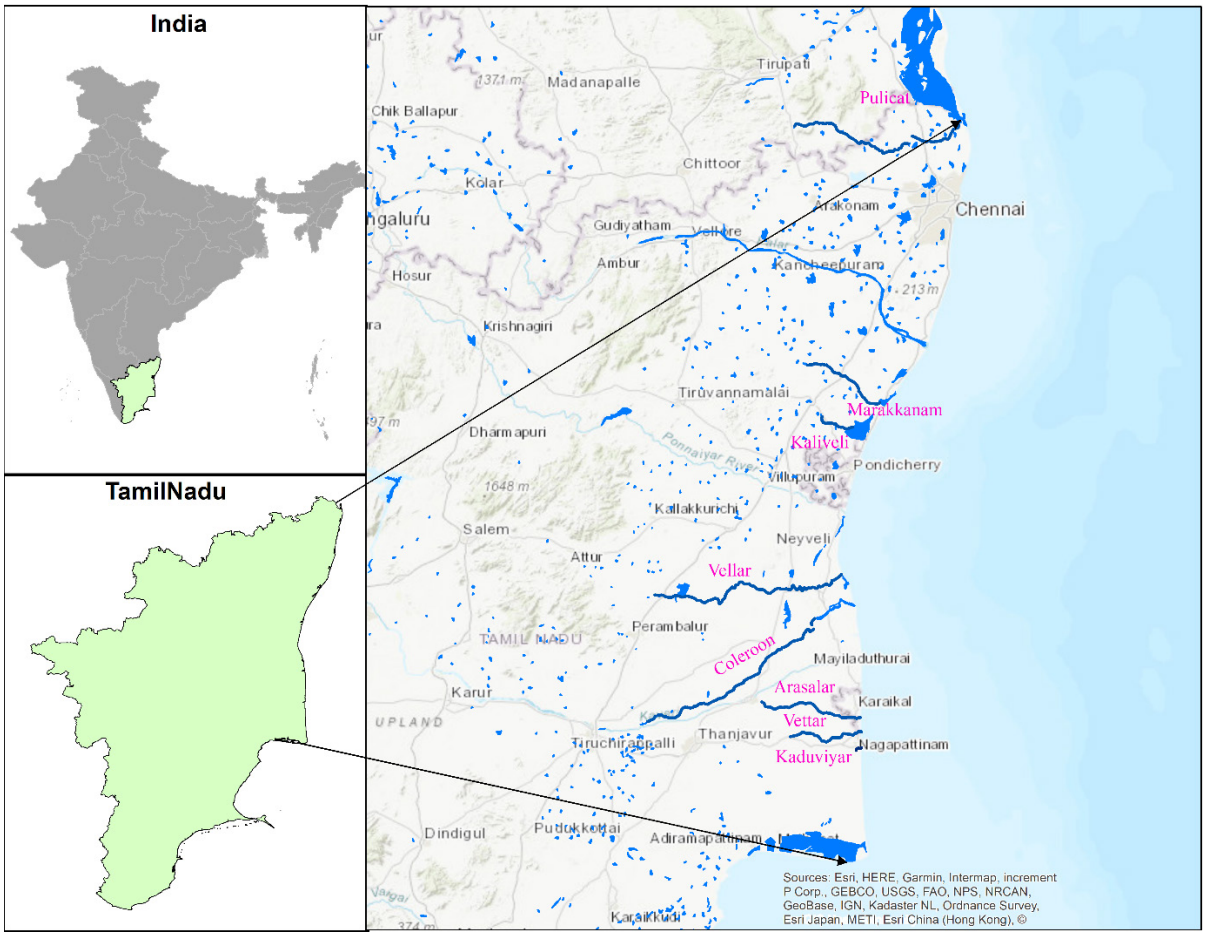

Figure 1. Map showing the localities of various estuaries studied for protozoan fauna.

ex., Karaikkal estuary, 06.i.2013; Mi-196, 6 ex., Arasalar estuary, 07.xii.2013; Mi-205, 4 ex., Coleron estuary, 08.xii.2013; Mi-229, 5 ex., Pulicat lake, 04.xii.2013; Mi-240, 4 ex., Vellar estuary, 20.vii.2014.

Diagnosis: Test dark brown, discoid in dorsal view and slightly oval in side view; height of the test about half the width; aperture oval and centrally located; pores present around the oral aperture; length of the test 118-124µm.

Distribution in India: Arunachal Pradesh, Assam, Jammu & Kashmir, Mizoram, Tamil Nadu (present record), Uttarakhand.

3. *Arcella conica* (Playfair, 1918)

Material examined: Mi-115, 7 ex., Pichavaram, 04.vii.2012; Mi-126, 4 ex., Coleron estuary, 04.vii.2012; Mi-161, 3 ex., Vellar estuary, 20.vi.2013; Mi-241, 2 ex., Vellar estuary, 20.vii.2014; Mi-283, 1ex., Vellar estuary, 20.vii.2014.

Diagnosis: Test oblique, slightly oval and angular in front view; mouth circular, proportionately small; diameter 92-98µm; aperture width 18 to 26µm.

Distribution in India: Andhra Pradesh, Arunachal Pradesh, Tamil Nadu (present record), West Bengal.

4. *Arcella crenulata* Deflandre, 1928

Material examined: Mi-141, 2 ex., Coleron estuary, 07.i.2013.

Diagnosis: Shell more or less circular with irregular outline; shell surface having many pores; aperture invaginated and margin irregular; test diameter 120-145µm and aperture diameter 19-31µm.

Distribution in India: Tamil Nadu (present record), West Bengal.

5. *Arcella dentata* Ehrenberg, 1830

Material examined: Mi-217, 3 ex., Kaduviyar estuary, 06.xii.2013.

Diagnosis: Test circular and pointed or dent like structures arising out evenly from the outer periphery of the test; test light brown in colour; aperture circular and central; diameter of test 115-128µm and aperture diameter 18-26µm.

Distribution in India: Tamil Nadu (present record), Uttar Pradesh, West Bengal.

6. *Arcella discoides* Ehrenberg, 1843

Material examined: Mi-116, 7 ex., Marakkanam estuary, 05.vii.2012; Mi-135, 5 ex., Vellar estuary, 08.i.2013; Mi-151, 5 ex., Karaikkal estuary, 19.vi.2013; Mi-162, 5 ex., Coleron estuary, 21.vi.2013; Mi-193, 7 ex., Arasalar estuary, 07.xii.2013;Mi-212, 4 ex., Coleron estuary, 08.xii.2013;Mi-231, 7 ex., Pulicat lake, 04.xii.2013;Mi-250, 8 ex., Kaliveli estuary, 21.vii.2014.

Diagnosis: Test brown in colour; outline smooth, circular in front view and planoconvex in lateral view; height of the dome about one fourth to one third of the diameter of the test; oral aperture at the centre, large and circular; diameter of the test 100-160µm and aperture diameter 40- 60µm.

Distribution in India: Assam, Andhra Pradesh, Arunachal Pradesh, Chandigarh, Himachal Pradesh, Kerala (Nilgiri Biosphere Reserve), Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tamil Nadu, Tripura, Uttarakhand, West Bengal.

7. *Arcella excavata* Cunningham, 1919

Material examined: Mi-117, 2 ex., Marakkanam estuary, 05.vii.2012; Mi-128, 1 ex., Coleron estuary, 04.vii.2012; Mi-206, 2 ex., Coleron estuary, 08.xii.2013; Mi-243, 2 ex., Coleron estuary, 20.vii.2014; Mi-251, 1 ex., Kaliveli estuary, 21.vii.2014; Mi-258, 2 ex., Arasalar estuary, 17.vii.2014.

Diagnosis: Test brown in colour; dorsal view slightly oval; aperture slightly oval in shape; aperture bordered by a thin lip; shell surface irregular with several pores; diameter of the test 54-62 µm; aperture diameter 18-22 µm.

Distribution in India: Tamil Nadu (present record). This forms new record for India.

8. *Arcella hemisphaerica* Perty, 1852

Material examined: Mi-197, 4 ex., Arasalar estuary, 07.xii.2013; Mi-207, 2 ex., Arasalar estuary, 08.xii.2013; Mi-213, 3ex., Coleron estuary, 08.xii.2013; Mi-232, 3 ex., Pulicat lake, 04.xii.2013; Mi-263, 2 ex., Vettar estuary, 20.vii.2014.

*Diagnosis***:** Shell brown and slightly oval in apertural view; hemispherical in lateral view; shell surface having slight depressions; aperture invaginated and bordered by a lip; more than 2 nuclei present; diameter of test 42-90 µm; aperture diameter 15 to 20 µm.

Distribution in India: Assam, Andhra Pradesh, Manipur, Odisha, Tamil Nadu (present record), West Bengal.

9. *Arcella megastoma* Penard, 1902

Material examined: Mi-129, 3 ex., Karaikkal estuary, 02.vii.2012; Mi-198, 5 ex., Kaduviyar estuary, 06.xii.2013; Mi-214, 3 ex., Coleron estuary, 06.xii.2013; Mi-219; 6 ex., Arasalar estuary, 06.xii.2013; Mi-253; 4 ex., Kaliveli estuary, 21.vii.2014; Mi-264; 3 ex., Vettar estuary, 20.vii.2014.

Diagnosis: Shell very flattened with a wide aperture; ratio diameter aperture/shell usually 0.45-0.53; with 2 to 200 nuclei; measurements 160-301 µm, diameter of aperture 87-190 µm.

Distribution in India: Andhra Pradesh, Himachal Pradesh, Tamil Nadu (present record).

Family CENTROPYXIDAE

10. *Centropyxis aculeata* (Ehrenberg, 1838)

Material examined: Mi-112, 2 ex., Coleron estuary, 02.vii.2012; Mi-125, 7ex., Kaliveli estuary, 05.vii.2012; Mi-130, 6 ex., Kaliveli estuary, 06.i.2013; Mi-142, 6 ex., Vellar estuary, 08.i.2013; Mi-147, 6 ex., Vellar estuary, 20.vi.2013; Mi-152, 4ex., Karaikkal estuary, 19.vi.2013; Mi-163, 4 ex., Coleron estuary, 21.vi.2013; Mi-244, 3 ex., Vellar estuary, 20.vii.2014.

Diagnosis: Test brown in colour, slightly compressed and encrusted with quartz crystals; fundus rounded and furnished with a few spines arranged in a single row; aperture semi-circular and eccentric; shell 97-158 µm in diameter.

Distribution in India: Assam, Andhra Pradesh, Arunachal Pradesh, Chandigarh, Himachal Pradesh, Kerala (Nilgiri

Biosphere Reserve), Manipur, Meghalaya, Mizoram, Nagaland, Rajasthan, Sikkim, Tamil Nadu, Tripura, West Bengal.

11. *Centropyxis aerophila* Deflandre, 1929

Material examined: Mi-148, 3 ex., Coleron estuary, 20.vi.2013.

Diagnosis: Test ovoid, yellow brown in colour, flanks of posterior part of the test arched outwards; aperture elliptical and eccentric; diameter of test 80 µm.

Distribution in India: Andhra Pradesh, Sikkim, Uttarakhand, Tamil Nadu, West Bengal.

12. *Centropyxis cassis* Deflandre, 1929

Material examined: Mi-338, 6 ex., Kaliveli estuary, 06.i.2013; Mi-450, 6 ex., Vellar estuary, 08.i.2013.

Diagnosis: Test elliptical, yellowish brown in colour, test formed of quartz particles; fundus rounded; flanks less arched; oral aperture semi circular and surrounded by large pebbles; test diameter 90-99 µm.

Distribution in India: Assam, Andhra Pradesh, Meghalaya, Odisha, Tamil Nadu (present record), Uttarakhand, West Bengal.

13. *Centropyxis constricta* Penard, 1890

Material examined: Mi-370, 6 ex., Vellar estuary, 08.i.2013; Mi-392, 6 ex., Vellar estuary, 20.vi.2013; Mi-401, 4 ex., Karaikkal estuary, 19.vi.2013.

Diagnosis: Test elliptical, grey in colour, posterior part arched, covered with sand stone particles; stony particles attached with posterior border; aperture eccentric and located near the anterior border of the test; diameter of test 110-140µm.

Distribution in India: Sikkim, Andhra Pradesh, Assam, Chandigarh, Himachal Pradesh, Meghalaya, Mizoram, Tamil Nadu, Tripura, Uttarakhand, West Bengal.

14. *Centropyxis delicatula* Penard, 1902

Material examined: Mi-350, 4 ex., Karaikkal estuary, 19.vi.2013; Mi-163, 4 ex., Coleron estuary, 21.vi.2013.

Diagnosis: Test yellowish brown in colour; the anterior part slightly circular, flattened in lateral view; aperture eccentric, test diameter 60-75 µm.

Distribution in India: Tamil Nadu (present record). This forms new record for India.

15. *Centropyxis discoides* Penard, 1902

Material examined: Mi-162, 3 ex., Coleron estuary, 08.xii.2013; Mi-356, 3ex., Pulicat lake, 04.xii.2013.

Diagnosis: Test semi-circular in outline, dish shaped in lateral view; shell encrusted with granular organic material, xenosomes scattered over the test; aperture eccentric and slightly oval; test diameter 120-290 µm.

Distribution in India: Tamil Nadu (present record). This forms new record for India.

16. *Centropyx isecornis* (Ehrenberg, 1841)

Material examined: Mi-326, 3 ex., Coleron estuary, 08.xii.2013.

Diagnosis: Test discoid, dark brown in colour; outline slightly irregular, spines absent and covered with quartz particles; flanks slightly arched and more elevated towards the posterior part; aperture eccentric and circular; test diameter 130-200µm.

Distribution in India: Assam, Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Kerala (Nilgiri Biosphere Reserve), Manipur, Meghalaya, Nagaland, Sikkim, Tamil Nadu (present record), West Bengal.

17. ***Centropyxis laevigata*** Penard, 1890

Material examined: Mi-149, 2 ex., Marakkanam estuary, 05.vii.2012.

Diagnosis: Test yellowish brown, almost circular in ventral view, usually slightly compressed in one side; test hemispherical in lateral view being boarder at posterior extremity; aperture eccentric, obliquely invaginated having apertural bridges, outer margin broadly rounded.

Distribution in India: Arunachal Pradesh, Mizoram, Sikkim, Tamil Nadu (present record), West Bengal.

18. *Centropyxis minuta* Deflandre, 1929

Material examined: Mi-153,4ex., Coleron estuary, 21.vi.2013.

Diagnosis: Test small, brown, more or less circular in ventral view, subspherical in lateral view with slightly more elevated posterior part; aperture circular, eccentric and invaginated, test encrusted with siliceous particles; test diameter 61µm.

Distribution in India: Assam, Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Manipur,

Meghalaya, Nagaland, Sikkim, Tamil Nadu, Tripura, West Bengal.

19. *Centropyxis oblonga* Deflandre, 1929

Material examined: Mi-322, 2ex., Marakkanam estuary, 20.vii.2014.

Diagnosis: Test yellowish brown, oblong in ventral view, in lateral view fundus of the test more elevated; aperture always elliptical and eccentric; test diameter 92-98µm.

Distribution in India: Assam, Meghalaya, Nagaland, Sikkim, Tamil Nadu (present record).

20. *Centropyxis orbicularis* Deflandre, 1929

Material examined: Mi-154, 6ex., Marakkanam estuary, 20.vii.2014.

Diagnosis: Shell brown and ovoid. Lateral view spherical, tapers towards the apical region. Shell surface usually rough, area around the aperture smooth; aperture invaginated, uneven in outline, two lateral internal extensions; test diameter 105-130µm.

Distribution in India: Andhra Pradesh, Himachal Pradesh, Tamil Nadu, Uttarakhand.

21. *Centropyxis spinosa* Cash, 1905

Material examined: Mi-137, 5 ex., Vellar estuary, 20.vii.2014.

Diagnosis: Test brown, more or less circular, dorsal side flat, furnished with variable number of spines irregularly distributed; aperture eccentric and circular; test with quartz crystals; shell 90-102 µm in diameter.

Distribution in India: Andhra Pradesh, Arunachal Pradesh, Chandigarh, Himachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tamil Nadu (present record), West Bengal.

22. *Cyclopyxis arcelloides* Deflandre, 1929.

Material examined: Mi-155, 5 ex., Marakkanam estuary, 22.vi.2013.

Diagnosis: Test brownish, circular in outer view; hemispherical in lateral view; siliceous scales scattered over the test; aperture central and circular and almost half the diameter of the test; test diameter 70-95µm.

Distribution in India: Andhra Pradesh, Arunachal Pradesh, Chandigarh, Himachal Pradesh, Manipur, Meghalaya, Mizoram, Sikkim, Tamil Nadu (present record), Uttarakhand, West Bengal.

23. *Cyclopyxis eurystoma* Deflandre, 1929

Material examined: Mi-150, 5 ex., Marakkanam estuary, 22.vi.2013.

Diagnosis: Test brown in colour, hemispherical in lateral view; elliptical in ventral view, encrusted with quartz particles; aperture central and circular and measures half the diameter of the test; diameter of the test 65-102µm.

Distribution in India: Assam, Arunachal Pradesh, Tamil Nadu (present record).

24. *Cyclopyxis kahli* (Deflandre, 1929)

Material examined: Mi-451, 5 ex., Marakkanam estuary, 22.vi.2013.

Diagnosis: Test yellowish, circular in ventral and dorsal view, in lateral view hemispherical; aperture centered, distinctly invaginated, roughly circular with irregular edge, xenosomes present; test diameter 70-85µm.

Distribution in India: Assam, Himachal Pradesh, Mizoram, Tamil Nadu (present record), Uttarakhand, West Bengal.

Family DIFFLUGIIDAE

25. *Difflugia avellana* Penard, 1890

Material examined: Mi-251, 3 ex., Arasalar estuary, 07.xii.2013.

Diagnosis: Test ovoid-elongate in front view and more or less pyriform in lateral view; posterior border rounded and flanks converging towards the aperture; aperture oval, test made up of siliceous particles

Distribution in India: Delhi, Meghalaya, Tamil Nadu (present record).

26. *Difflugia acuminata* Ehrenberg, 1838

Material examined: Mi-281, 5 ex., Vellar estuary, 20.vi.2013.

Diagnosis: Shell light brown, cylindrical, slightly tapering towards the apertural end; posterior end pointed or with a hollow and open tube or spine; shell composed of mineral particles.

Distribution in India: Assam, Andhra Pradesh, Kerala

(Nilgiri Biosphere Reserve), Meghalaya, Rajasthan, Tamil Nadu, Tripura, West Bengal.

27. *Difflugia amphora* (Leidy, 1874) Penard, 1902

Material examined: Mi-200; 3 ex., Kaduviyar estuary, 06.xii.2013.

Diagnosis: Test brown in colour and pyriform, circular in cross-section, composed of quartz crystals throughout the test; fundus rounded; test diameter 115-162 µm.

Distribution in India: Andhra Pradesh, Tamil Nadu (present record).

28. ***Difflugia binucleata*** Penard, 1902

Material examined: Mi-122, 3 ex., Marakkanam estuary, 22.vi.2013.

Diagnosis: Test dark brown, ovoid; fragments of diatoms scattered over the test; two vesicular nuclei present; measurements 122 - 160 µm.

Distribution in India: Tamil Nadu (present record). This forms new record for India.

29. *Difflugia corona* Wallich, 1864

Material examined: Mi-156, 2ex., Marakkanam estuary, 07.xii.2013; Mi-194, 7 ex., Arasalar estuary, 07.xii.2013; Mi-201, 6ex., Arasalar estuary, 08.xii.2013; Mi-216, 6ex., Coleron estuary, 08.xii.2013; Mi-222, 6ex., Kaduviyar estuary, 06.xii.2013; Mi-248, 4ex., Vellar estuary, 20.vii.2013.

Diagnosis: Test almost spherical; narrowing towards pseudostome; widening towards the base with the presence of 5-10 spines; test formed by quartz crystals; aperture large and occupies half the diameter of the test; measurements 73-125 µm.

Distribution in India: Assam, Andhra Pradesh, Kerala (Nilgiri Biosphere Reserve), Tamil Nadu (present record), Tripura, West Bengal.

30. *Difflugia geosphaira* Ogden, 1991

Material examined: Mi-113, 2 ex., Coleron estuary, 02.vii.2012; Mi-274, 2 ex., Vellar estuary, 20.vii.2014.

Diagnosis: Shell dark brown, more or less ovoid, apertural rim is very prominenet; aperture large, circular; nucleus with many peripheral nucleoli, three contractile vacuoles; measurements: 51-85 µm long.

Distribution in India: Tamil Nadu (present record). This forms new record for India.

31. *Difflugia globulosa* (Dujardin, 1837) Penard, 1902

Material examined: Mi-202, 2 ex., Arasalar estuary, 07.xii.2013; Mi-223, 4ex., Kaduviyar estuary, 06.xii.2013; Mi-234,3ex., Vettar estuary, 04.xii.2013; Mi-268,4ex., Arasalar estuary, 17.vii.2014; Mi-279, 4ex., Vellar estuary, 20.vii.2014; Mi-300, 4ex., Kaduviyar estuary, 11.x.2015.

Diagnosis: Test composed of prominent quartz particles; brown, hemispherical; aperture circular, surrounded by small sand particles; diameter of shell 81-118 µm.

Distribution in India: Andhra Pradesh, Rajasthan, Meghalaya, Tamil Nadu (present record), West Bengal.

32. *Difflugia gramen* Penard, 1902

Material examined: Mi-123, 2 ex., Marakkanam estuary, 05.vii.2012; Mi-138, 4 ex., Vellar estuary, 08.i.2013; Mi-224, 3 ex., Kaduviyar estuary, 06.xii.2013; Mi-249, 4 ex., Vellar estuary, 20.vii.2014; Mi-269, 4 ex., Arasalar estuary, 13.x.2015.

Diagnosis: Test ovoid, brown in colour, not compressed, composed of mineral particles of various sizes, surface of test more or less rough in appearance; aperture with three lobes having small ridges, often surrounded with a row of small granules; aperture sometimes with collar; diameter of test 61-125 µm.

Distribution in India: Andhra Pradesh, Tamil Nadu (present record), West Bengal.

33. *Difflugia lebes* Penard, 1899

Material examined: Mi-131, 2 ex., Karaikkal estuary, 06.i.2013.

Diagnosis: Test light brown in colour; semi-circular test; aperture large and covers entire edge of the test; test covered with siliceous particles; collar straight; more number of nuclei; test dimension 150-190µm.

Distribution in India: Andhra Pradesh, Tamil Nadu (present record).

34. *Difflugia lithophila* Penard, 1902

Material examined: Mi-132, 2 ex., Karaikkal estuary, 06.i.2013; Mi-138, 4 ex., Vellar estuary, 06.xi.2015.

Diagnosis: Test sub-globular, pseudostome drawn out in the form of short collar; pseudostome circular; test covered with stony particles; test diameter 84-121 µm.

Distribution in India: Chandigarh, Meghalaya, Tamil Nadu (present record), Tripura, West Bengal.

35. *Difflugia limnetica* Penard, 1869

Material examined: Mi-123, 2 ex., Marakkanam estuary, 05.vii.2012.

Diagnosis: Test ovoid globular; flanks slightly outward and spread on both sides, mouth at the anterior end; test provided with large quartz particles; surface appears rough; dimension 82-102µm.

Distribution in India: West Bengal, Tamil Nadu (present record).

36. *Difflugia lucida* Penard, 1890

Material examined: Mi-226, 2 ex., Arasalar estuary, 07.xii.2013.

Diagnosis: Test light brown, elongate, transparent, slightly compressed, covered with quartz crystals; aperture at the extreme anterior end; apertural plug present at the margin of the aperture; dimension 62-78µm.

Distribution in India: Nagaland, Sikkim, Tamil Nadu, Uttarakhand.

37. *Difflugia lobostoma* Leidy, 1879

Material examined: Mi-249, 2 ex., Vellar estuary, 20.vii.2014.

Diagnosis: Test ovoid, pseudostome usually quadrilobed, sometimes trilobed, pseudostome without collar, test covered with quartz crystals.

Distribution in India: Andhra Pradesh, Arunachal Pradesh, Chandigarh, Himachal Pradesh, Kerala (Nilgiri Biosphere Reserve), Manipur, Meghalaya, Nagaland, Rajasthan, Tamil Nadu, Tripura, West Bengal.

38. *Difflugia oblonga* Ehrenberg, 1838

Material examined: Mi-340, 2 ex., Vellar estuary, 20.vii.2014.

Diagnosis: Test typically oblong with circular base; composed of big angular quartz crystals; pseudostome circular; dimension of test 90-115µm.

Distribution in India: Assam, Himachal Pradesh, Meghalaya, Sikkim, Tamil Nadu, West Bengal.

39. *Difflugia tuberculata* Wallich, 1864

Material examined: Mi-133, 2 ex., Karaikkal estuary, 06.i.2013.

Diagnosis: Test ovoidal with wide base; pseudostome hexagonal in outline with obtuse angles surrounded by a short collar; test yellowish or chocolate brown, covered with tuberclesor disc shaped protruberances and composed of small platelets perfectly uniting the tubercles; dimension 124-138µm.

Distribution in India: Meghalaya, Nagaland, Tamil Nadu, West Bengal.

Family HYALOSPHENIIDAE

40. *Argynnia caudata* (Leidy, 1879)

Material examined: Mi-183, 2 ex., Marakkanam estuary, 07.xii.2013.

Diagnosis: Shell ovoid with a rounded or angulous fundus, compressed conical processes projecting outwards near fundus; test covered with circular platetets, sometimes joining and rarely overlapping; aperture oval; dimension of test 65-88µm.

Distribution in India: Assam, Sikkim, Tamil Nadu (present record).

41. *Argynnia dentistoma* (Penard, 1890)

Material examined: Mi-255, 2 ex., Kaliveli estuary, 21.vii.2014.

Diagnosis: Test ovoid, compressed, without a prominent neck, aperture terminal and margin of the aperture crenulate; test covered with transparent and oval small platelets of uniform size; made up of siliceous substance; test dimension 65-95µm.

Distribution in India: Assam, Arunachal Pradesh, Meghalaya, Sikkim, Tamil Nadu, West Bengal.

42. *Gibbocarina galeata* (Penard, 1890) Kosakyan *et al*., 2016

Material examined: Mi-166, 2 ex., Coleron estuary, 21.vii.2013.

Diagnosis: Test light brown in colour, on either side of the lateral margin of the pseudostome small pore present; test composed of oval plates; aperture oval; dimension of test

115-155µm.

Distribution in India: Himachal Pradesh, Tamil Nadu.

43. *Padungiella lageniformis* (Penard, 1890) Kosakyan *et al*., 2016

Material examined: Mi-105, 2 ex., Coleron estuary, 2.vii.2012.

Diagnosis: Test typically flask shaped; aperture arched and covered with thick organic collar; in lateral view test vase shaped; test covered with rounded plates of various sizes; dimension of test 130-140µm.

Distribution in India: Sikkim, Mizoram, Tamil Nadu (present record).

44. *Padaungiella wailesi* (Deflandre, 1936) Kosakyan *et al*., 2016

Material examined: Mi-97, 2 ex., Marakkanam estuary, 5.vii.2012.

Diagnosis: Test flask-shaped; aperture slightly arched and bordered by thickened organic collar; in lateral view aperture moderately notched and test vase-shaped but slightly compressed; test covered with platelets of mixed sizes.

Distribution in India: Himachal Pradesh, Sikkim, Tamil Nadu.

Family EUGLYPHIDAE

45. *Euglypha acanthophora* (Ehrenberg, 1841)

Material examined: Mi-107, 2 ex., Coleron estuary, 2.vii.2012; Mi-256, 2 ex., Kaliveli estuary, 21.vii.2014.

Diagnosis: Test ovoid elongated, aperture circular and bordered by apertural platelets; test platelets elliptical, some platelets prolonged into 4-6 long spines; test dimension 65-75µm.

Distribution in India: Andhra Pradesh, Assam, Manipur, Meghalaya, Nagaland, Tamil Nadu, West Bengal.

46. *Euglypha cristata* Leidy, 1874

Material examined: Mi-152, 4 ex., Kaduviyar estuary, 19.vi.2013.

Diagnosis: Test brown, slightly compressed, fundus hemispherical, provided with a bunch of 3-6 curved spines; aperture circular bordered by a single row of denticulated platelets; dimension of test 45-65µm.

Distribution in India: Tripura, Tamil Nadu.

Phylum CILIOPHORA

Class SPIROTRICHEA

Order TINTINNIDA

Family CODNELLIDAE

47. *Tintinnopsis ampla* Hada, 1937

Material examined: Mi-178, 3 ex., Marakkanam estuary, 22.vi.2013; Mi-284, 10 ex., Kaliveli estuary, 20.vii.2014.

Diagnosis: Loricabowl shaped and large, oral margin slightly tapering; aboral horns present; aboral end pointed and a small aboral horn present.

Distribution in India: Tamil Nadu, West Bengal.

48. *Tintinnopsis angusta* Meunier, 1910

Material examined: Mi-208, 2 ex., Coleron estuary, 8.xii.2013.

Diagnosis: Lorica short; oral diameter more than half the diameter of bowl; length 5-65µm.

Distribution in India: Odisha, Tamil Nadu, West Bengal.

49. *Tintinnopsis dadayi* Kofoid, 1905

Material examined: Mi-179, 3 ex., Kaliveli estuary, 22.vi.2013.

Diagnosis: Lorica elongated and compressed; a cylindrical collar and a globose bowl; irregular oral rim; aboral end slightly pointed; total length 45-65µm.

Distribution in India: Kerala, Tamil Nadu, West Bengal.

50. *Tintinnopsis directa* Hada, 1932

Material examined: Mi-180, 4 ex., Coleron estuary, 21.vi.2013; Mi-209, 2 ex., Coleron estuary, 08.xii.2013; Mi-301, 6 ex., Kaduviyar estuary, 11.x.2015.

Diagnosis: Lorica straight till the bulb of the aboral end; oral rim irregular; suboral region conical; posterior region subspherical with a rounded aboral end.

Distribution in India: Odisha, Tamil Nadu, West Bengal.

51. *Tintinnopsis gracilis* Kofoid & Campbell, 1929

Material examined: Mi-170, 4 ex., Kaliveli estuary, 22.vi.2013; Mi-181, 3 ex., Coleron estuary, 21.vi.2013; Mi-288, 15 ex., Marakkanam estuary, 20.vii.2014; Mi-299, 3 ex., Vettar estuary, 06.xii.2015.

Diagnosis: Lorica finger-shaped, oral margins mooth; lorica swollen in the posterior one-third of the bowl; aboral end convex conical with a blunt distal end; length 95-105 µm.

Distribution in India: Odisha, Tamil Nadu, West Bengal.

52. *Tintinnopsis karajacensis* Brandt, 1896

Material examined: Mi-210., 2 ex., Coleron estuary, 08.xii.2013; Mi-303., 4 ex., Kaduviyar estuary, 11.x.2015.

Diagnosis: Lorica capsular in shape, bowl cylindrical, oral rim rough; aboral end rounded; length 95-140µm.

Distribution in India: Tamil Nadu, West Bengal.

53. *Tintinnopsis nana* Lohmann, 1908

Material examined: Mi-182, 6 ex., Marakkanam estuary, 22.vi.2013; Mi-312, 4 ex., Kaduviyar estuary, 11.x.2015.

Diagnosis: Lorica elongated and pointed at aboral end; oral aperture rim rough and irregular with thin wall; length 45-55µm.

Distribution in India: Odisha, Tamil Nadu, West Bengal.

54. *Tintinnopsis parvula* Jorgensen, 1912

Material examined: Mi-171, 3 ex., Kaliveli estuary, 22.vi.2013; Mi-183, 4 ex., Marakkanam estuary, 22. vi. 2013; Mi-306, 4 ex., Kaduviyar estuary, 11.x.2015.

Diagnosis: Lorica small in size; oral end broadened; aboral end pointed; length 55-65µm.

Distribution in India: Odisha, Tamil Nadu.

55. *Tintinnopsis rotundata* Jorgensen, 1899

Material examined: Mi-184, 4 ex., Kaliveli estuary, 22.vi.2013.

Diagnosis: Lorica small and cylindrical; aboral end hemispherical; oral margin rough and serrated; wall of the lorica thin; length 50-75µm.

Distribution in India: Tamil Nadu, West Bengal.

56. *Tintinnopsis schotti* Brandt, 1906

Material examined: Mi-172, 4 exs., Kaliveli estuary, 22. vi. 2013.

Diagnosis: Lorica pot-shaped, broadened towards the aboral end below the middle part; aboral region broadly convex conical (96-120 degrees) to a blunt distal end; length 90-105µm.

Distribution in India: Odisha, Tamil Nadu, West Bengal.

57. *Tintinnopsis tocantinensis* Kofoid & Campbell, 1929

Material examined: Mi-173, 5 ex., Kaliveli estuary, 22.vi.2013;Mi-185, 6 ex., Kaliveli estuary, 22.vi.2013;Mi-190, 4 ex., Arasalar estuary, 07. xii. 2013; Mi-289, 11 ex., Marakkanam estuary, 20.vii.2014; Mi-308, 6 ex., Kaduviyar estuary, 11.x.2015.

Diagnosis: Lorica long and cylindrical, distal end bulb shaped and ends with a stout pointed horn; length 112µm.

Distribution in India: Odisha, West Bengal, Tamil Nadu.

58. *Tintinnopsis undella* Meunier, 1910

Material examined: Mi-174, 3 ex., Kaliveli estuary, 22.vi.2013; Mi-186, 3 ex., Marakkanam estuary, 22.vi.2013; Mi-290, 10 ex., Marakkanam estuary, 20.vii.2014.

Diagnosis: Lorica vase shaped; expanded and widened in the oral region; length 90µm; oral diameter 30µm.

Distribution in India: Tamil Nadu, West Bengal.

Discussion

Estuaries are very diverse and productive ecosystems which have not been studied seriously for protist diversity in India and in view of this a study has been made to explore the freeliving protist diversity in some estuaries along the coastal zones of Tamil Nadu. The study revealed the occurrence of 58 species of freeliving protozoans coming under 9 genera and 6 families of which 25 species are new records from Tamil Nadu and it is remarkable that 5 species are new additions to Indian testate fauna *viz*., *Arcella excavata* Cunningham 1919, *Centropyxis delicatula* Penard 1902, *Centropyxis discoides* Penard 1902, *Difflugia binucleata* Penard 1902 and *Difflugia geosphaira* Ogden 1991. The present estimate of free-living protozoans recorded from India including the estuarine species is about 1600 of which 185 species belong to testate amoebae and the rest are other groups.

The number of protist species recorded from Tamil Nadu is very low which depicts a meagre diversity of species from such a highly diversified ecosystem; however the actual diversity must be far above the recorded number of species. It is suggested that more taxonomists should come forward and appropriate and serious studies should be done to enrich the diversity of free-living protists along various estuaries of Tamil Nadu.

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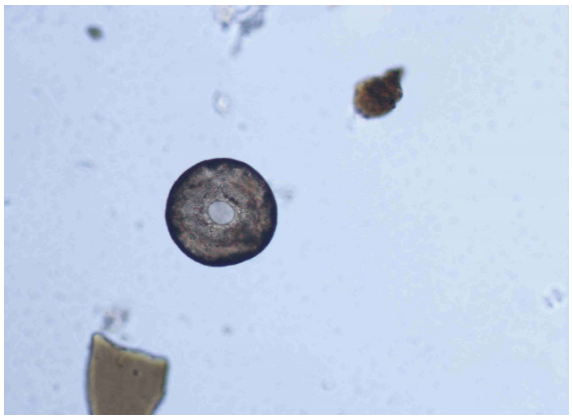
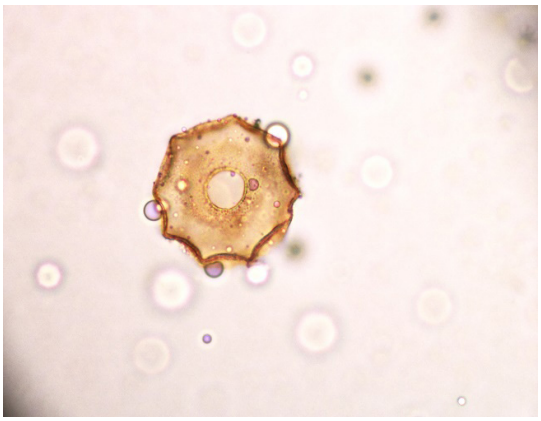
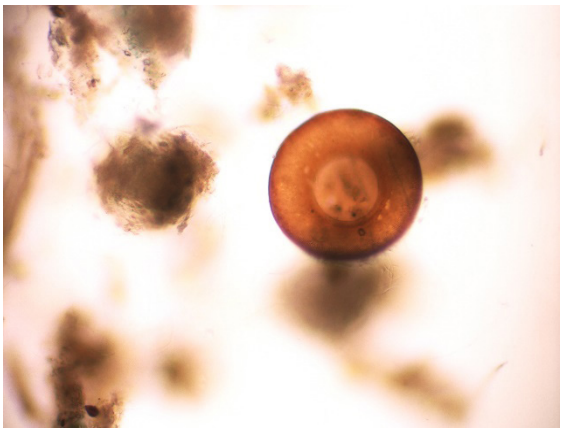


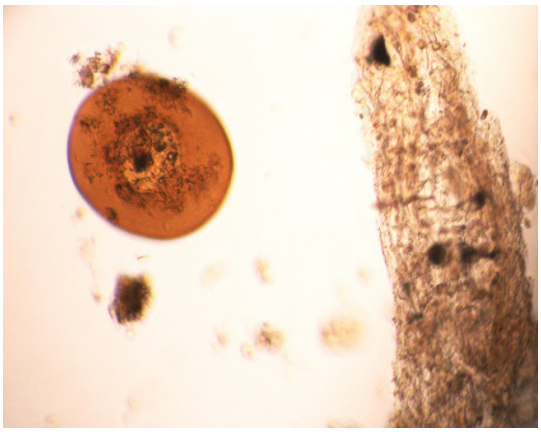
Arcella arenaria Greeff, 1866



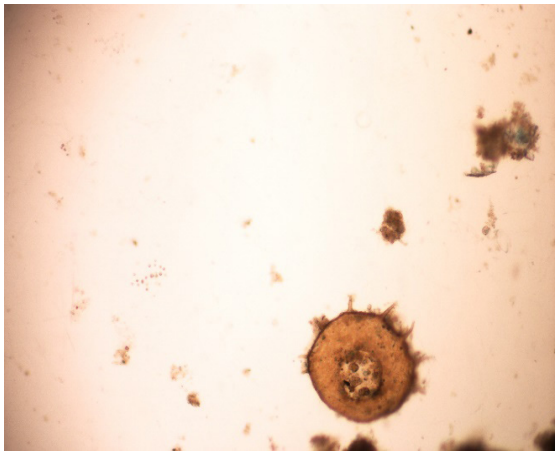
Arcella dentata Ehrenberg, 1830



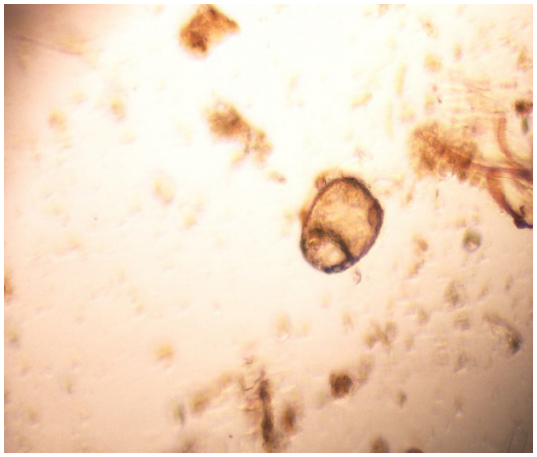
Arcella discoides Ehrenberg, 1843



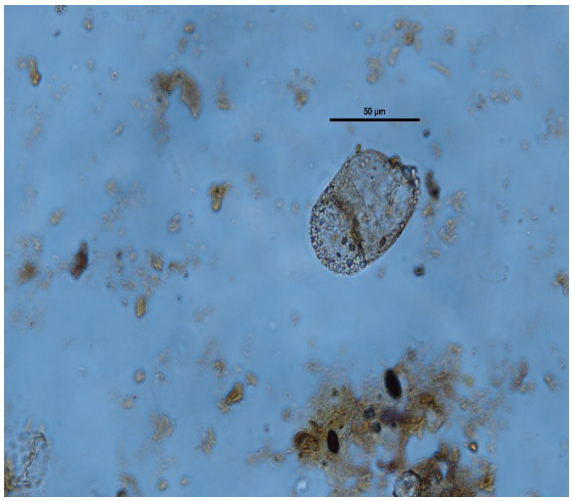
Arcella excavata Cunningham, 1919



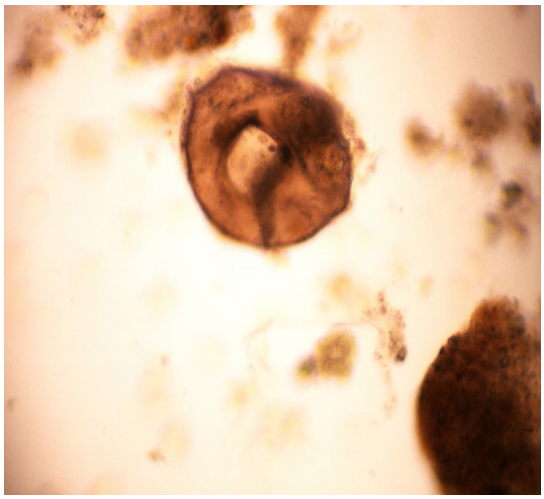
Centropyxis aculeata Ehrenberg, 1838



Centropyxis aerophila Deflandre, 1929

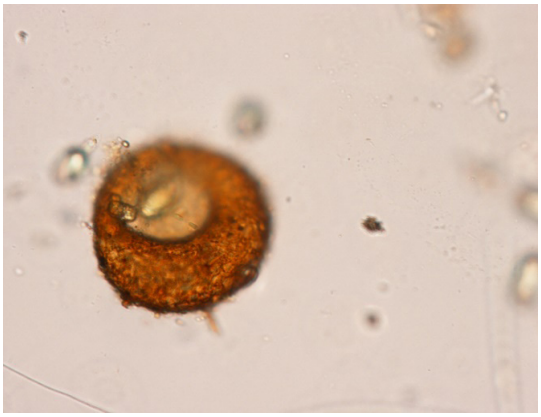


Centropyxis constricta Penard, 1890

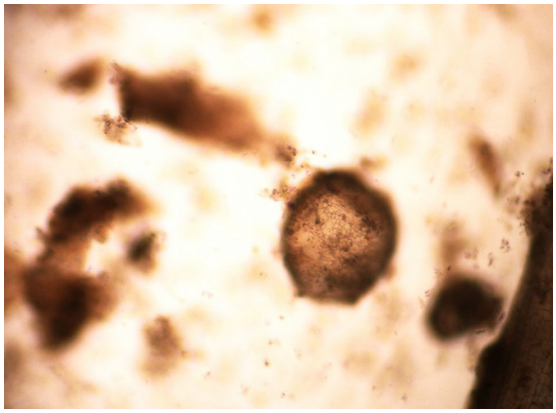


Centropyxis discoides Penard, 1902

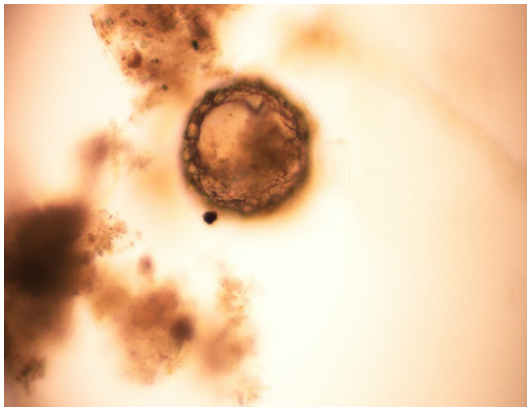
Centropyxis laevigata Penard, 1890



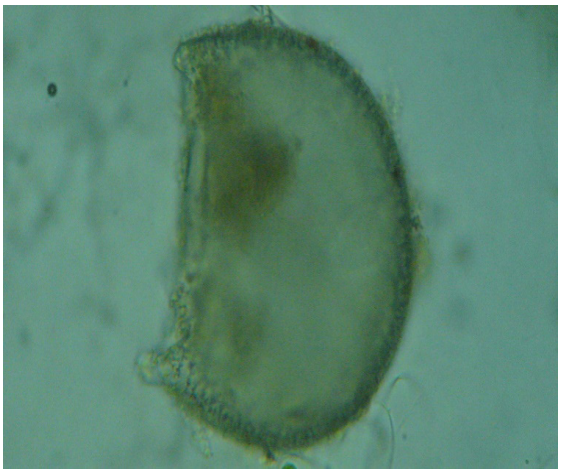
Centropyxis orbicularis Deflandre, 1929



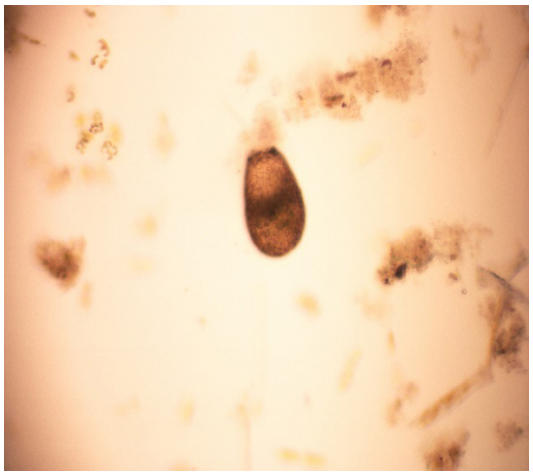
Difflugia binucleata Penard, 1902



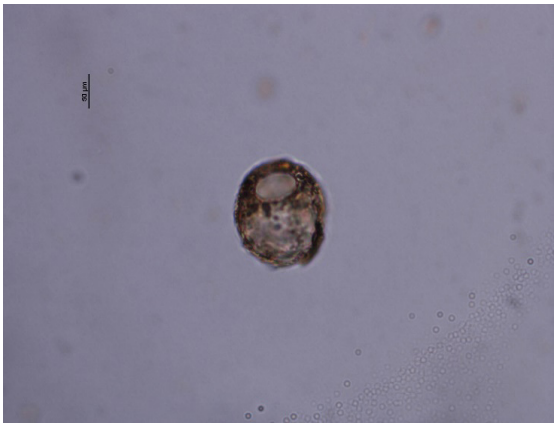
Difflugia geosphaira Ogden, 1991



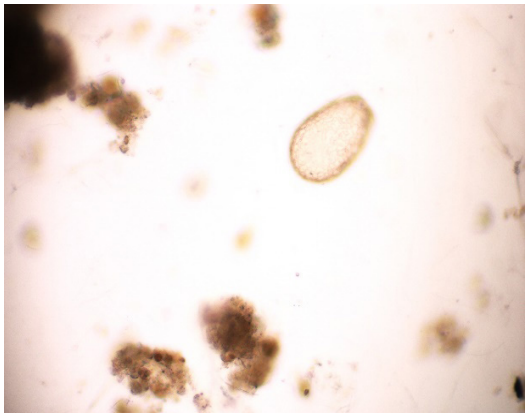
Difflugia lebes Penard, 1890



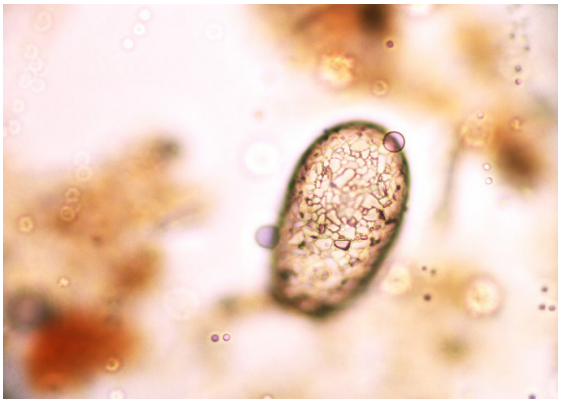
Difflugia lithophila Penard, 1902



Difflugia oblonga Ehrenberg, 1838

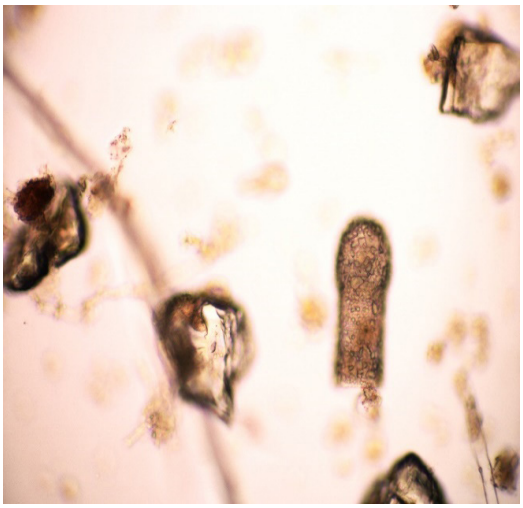


Difflugia tuberculata Wallich 1864

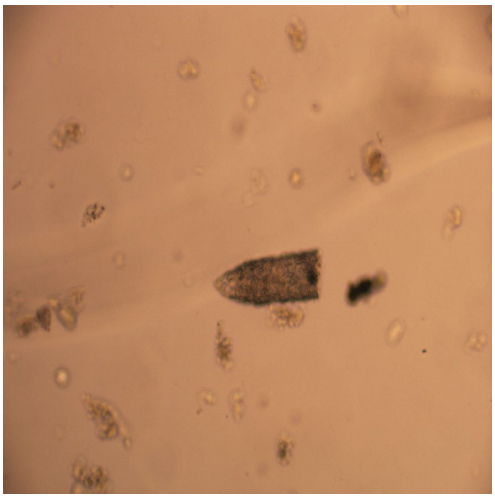


Argynnia dentistoma (Penard, 1890)

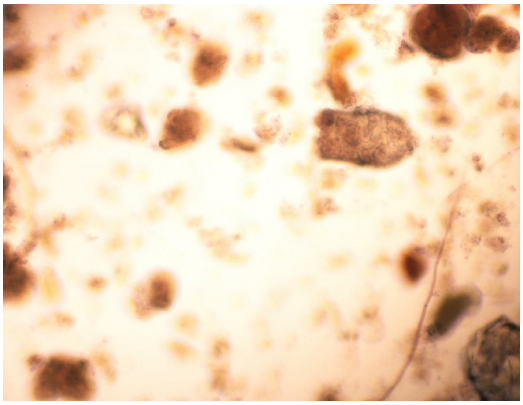
Tintinnopsis dadayi Kofoid, 1905



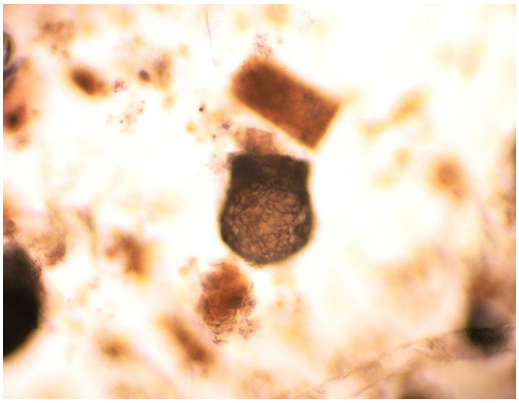
Tintinnopsis directa Hada, 1932



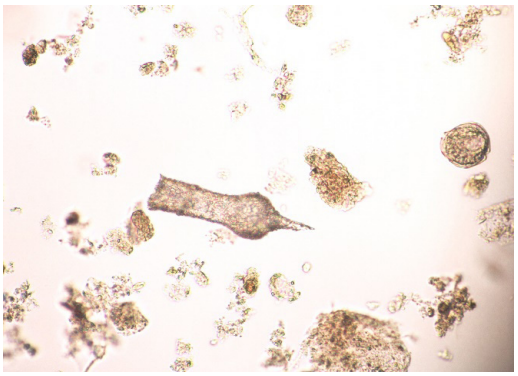
Tintinnopsis gracilis Kofoid & Campbell, 1929



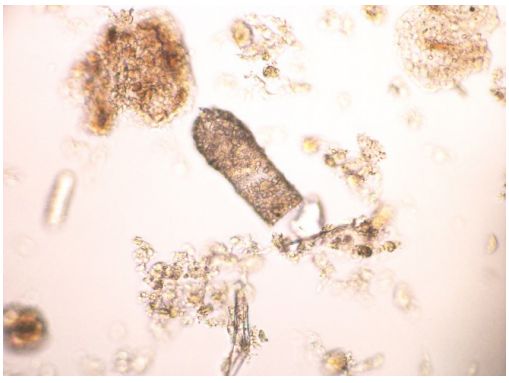
Tintinnopsis parvula Jorgensen, 1912



Tintinnopsis schotti Brandt, 1906



Tintinnopsis tocantinensis Kofoid & Campbell 1929



Tintinnopsis undella Meunier, 1910